Dispatches

# Evolution: Rooting the Eukaryotic Tree of Life

**The root of the eukaryotic tree is a major unresolved question in evolutionary biology. A recent study marshals mitochondrial genes to place that root between the enigmatic Excavates and all other eukaryotes, providing an interesting new perspective on early eukaryotic evolution.**

**Tom A. Williams**

Eukaryotes are the group of organisms whose cells contain a mitochondrion and a nucleus. Although we often think of eukaryotes as comprising animals, plants, fungi, and the single-celled protists, molecular phylogenetics has turned that view on its head [1]. At the level of the genome, eukaryotic biodiversity is largely microbial, with each of the familiar multicellular groups arising independently from unicellular ancestors [2]. In this emerging view, which is based mainly on phylogenetic analyses of large sets of broadly distributed genes [3–5], most eukaryotes can be classified into one of three major lineages. These are the Amorphea (animals, fungi, and Amoebozoans such as the slime mould *Dictyostelium*), the Diaphoretickes (including plants and most other photosynthetic eukaryotes), and the Excavates — a diverse group of unicellular eukaryotes that includes important parasites such as *Giardia*, *Trichomonas*, and *Trypanosoma.* Despite this rapid progress, some of the most interesting questions about eukaryotic evolution remain unresolved. Foremost among these is the position of the root of the eukaryotic tree, a critical point about which there is currently little consensus. A new study by He *et al.* [6] reported in this issue of *Current Biology* represents an important contribution to this ongoing debate.

The root of a phylogenetic tree is fundamental to its biological interpretation, defining the starting point and polarizing the subsequent divergence events within the tree. For the eukaryotic tree, the root position is critical for identifying the genes and traits that may have been present in the ancestral eukaryote, for tracing the evolution of these traits throughout the eukaryotic radiation, and for establishing the deep relationships among the major eukaryotic groups. The root of the eukaryotes has been studied intensively in recent years, although as yet no consensus appears in sight. Published analyses have placed the root within the Amorphea [7], between the Amorphea and all other eukaryotes [8], between the Archaeplastida (plants) and all other eukaryotes [9], or within the Excavates [10] — all with rather different implications for the history and the process of eukaryotic evolution.

One reason for this lack of agreement may be the intrinsic difficulty of rooting the eukaryotic tree. The great evolutionary distance that separates eukaryotes from their closest prokaryotic relatives makes standard phylogenetic rooting approaches unreliable and prone to artefact. This has led authors to explore alternative approaches, such as using the information from gene duplications and losses [7] or slowly evolving molecular characters such as gene fusions [9] to root the tree. The problem is that when these alternative strategies disagree, it is difficult to determine the best approach. As an added difficulty, the interior branches of the eukaryotic tree separating the major groups often appear to be rather short, perhaps suggesting that the Amorphea, Diaphoretickes and Excavates diverged from each other over a relatively short period of time following the origin of eukaryotes — the so-called ‘big bang’ of eukaryotic evolution [11,12].

Despite these difficulties in inferring the root, recent discoveries have fleshed out our understanding of eukaryotic origins and the ancestral eukaryote. The balance of evidence now favours a symbiogenic origin for the eukaryotic cell, in which an Alpha-proteobacterium — the ancestor of the mitochondrion — formed a partnership with an Archaeon, whose trace in modern eukaryotes is found mainly in the genetic apparatus of DNA synthesis, transcription and, particularly, translation [13]. This scenario implies that the mitochondrion was gained before the radiation of all known eukaryotes, suggesting that mitochondrial genes might provide an interesting source of information about the eukaryotic root. The idea is that, since mitochondria are descended from Bacteria, trees of mitochondrial genes can be rooted on the bacterial branch, polarizing the relationships within the eukaryotic portion of the tree — an approach pioneered by Derelle and Lang [8] and developed in the recent study of He *et al.* [6]. While this work is unlikely to settle the debate for good, He *et al.* employ state-of-the-art methods, and their analyses focus attention on the most important challenges remaining in this field.

As a starting point for their analyses, He *et al.* [6] screened genomes from the Amorphea, Diaphoretickes and Excavates for mitochondrial genes. They identified 37 genes which were found in all, or almost all, eukaryotes, and whose closest prokaryotic homologues were among the Bacteria. They found that the protein products of most of these genes were targeted to the mitochondria of modern eukaryotes, consistent with the idea that the genes originally entered the eukaryotic lineage with the ancestor of the mitochondrion. Note that these genes are often not encoded by the mitochondrial genome, because many mitochondrial genes have been transferred to the nucleus during eukaryotic evolution [14]. Importantly, He *et al.* were able to show that their set of genes contained a consistent phylogenetic signal within the eukaryotes — that is, that they generally agreed on the structure of the eukaryotic tree. Since each gene on its own did not provide much rooting information, the authors combined all 37 genes and inferred a single tree. Assuming that the mitochondrion was acquired before the diversification of the main eukaryotic lineages, this tree could then be rooted on the branch leading to the bacterial outgroups (Figure 1). Following this logic, He *et al.*

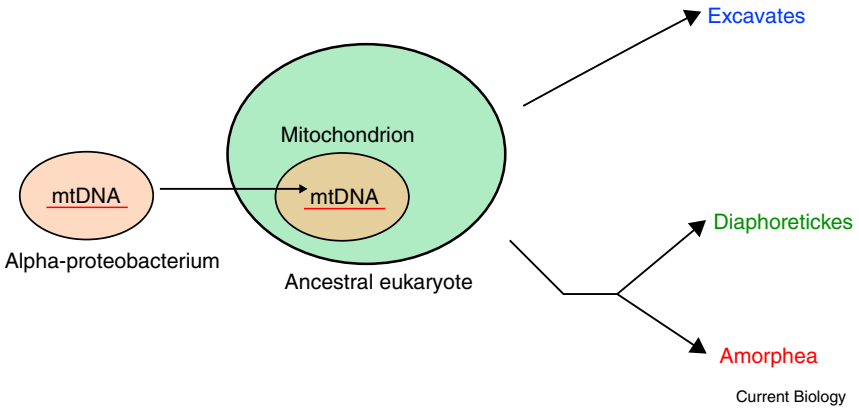


Figure 1. Mitochondrial genes root the eukaryotic tree.

Genes that entered the eukaryotic lineage with the mitochondrial endosymbiont trace the subsequent evolutionary diversification of the eukaryotes. The recent study of He *et al.* [6] suggests that one of the deepest splits lies between the Excavates and all other eukaryotes.

rooted the tree between the Excavates and all other eukaryotes.

If correct, an Excavate root would reinforce the astonishing biodiversity of microbial eukaryotes by placing all of the familiar multicellular groups — animals, plants, and fungi — on one side of the eukaryotic tree. It would also support an emerging view of the last common eukaryotic ancestor as a relatively complex single-celled organism that already possessed a gene repertoire comparable in size and complexity to modern eukaryotes [15,16].

But with a number of incompatible rootings now proposed for the eukaryotic tree, why should this new root be preferred to any of the alternatives? One reason might be the apparent robustness of the rooting signal from the 37 genes analyzed by He *et al.* When the authors compared support for the Excavate root with two of the leading alternatives — a root on the branch leading to Amorphea (the 'unikont–bikont' root) or to Diaphoretickes — they found that both alternatives could be strongly rejected, even using only small subsamples of their combined alignment. This suggests a strong signal for the Excavate root, at least from the set of mitochondrial genes considered. But the selection of those genes may represent a potential caveat of this study, or at least a subject for further work. A recent study by Derelle and Lang [8] applied a similar strategy to He *et al.*, using mitochondrial genes to root the eukaryotic tree — but their analyses instead supported a root on the amorphean branch. The difference comes down to the definition of mitochondrial genes: while He *et al.* emphasised bacterial genes that contain a congruent phylogenetic signal and are broadly conserved across eukaryotes, Derelle and Lang screened for eukaryotic genes of alpha-proteobacterial affinity, based on the idea that the mitochondrion is descended from an Alpha-proteobacterium. As a result of these different criteria, there was only moderate overlap between the genes used in the two studies. In particular, not all of the genes analyzed by He *et al.* are alpha-proteobacterial, with some tracing their ancestry to other bacterial groups. This does not necessarily rule out a mitochondrial origin, because the ancestral mitochondrial genome likely contained genes horizontally acquired from other prokaryotic groups, as is observed for the genomes of contemporary Alpha-proteobacteria [17]; in principle, genes obtained from other Bacteria could also be used to root the tree if acquired before the radiation of eukaryotes. He *et al.* argue that their approach allowed them to better detect and remove mitochondrial genes that had undergone horizontal transfer during eukaryotic evolution, which might interfere with root inference if included in the analysis.

This is an exciting time for research into eukaryotic origins. The new study of He *et al.* is a thorough and interesting contribution to the rooting question, although it is unlikely to provide the final word on this topic. The fact that similar approaches can produce very different results indicates that we still have much to learn about the earliest period of eukaryotic evolution, and will surely stimulate further debate.

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Institute for Cell and Molecular Biosciences, University of Newcastle, Newcastle upon Tyne NE2 4HH, UK.
E-mail: tom.williams2@ncl.ac.uk

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